

# Recipes Research

## Study on Drug Property Differences of Shexiang (Moschus) and Bingpian (Borneolum Synthcticum) Based on Analysis of Biothermodynamics

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**Objective:** To study the drug property differences of Shexiang (Moschus) and Bingpian (Borneolum Synthcticum) with biothermodynamics, and to verify the objectivity and authenticity of the drug property.

**Methods:** The growth-thermogram curves of *Escherichia coli* (*E. coli*) affected by Shexiang (Moschus) and Bingpian (Borneolum Synthcticum) at different concentrations were determined with microcalorimetry, and the power-time curves (thermogram curves) of *E. coli* metabolism and characteristic parameters, such as growth rate constant ( $k$ ), maximum output power ( $P_m$ ), peak time ( $t_p$ ), total heat-output ( $Q_t$ ), etc. were analyzed with the principal component analysis (PCA) to find the close correlative parameters, so as to objectively reflect the drug property differences of Shexiang (Moschus) and Bingpian (Borneolum Synthcticum).

**Results:** The values of  $P_2$  in the second exponential growth phase increased with the increase of the concentrations of Shexiang (Moschus) and Bingpian (Borneolum Synthcticum), and the  $P_2$  of Shexiang (Moschus) was larger than that of Bingpian (Borneolum Synthcticum);  $Q_2$  increased with the increase of the concentrations of Shexiang (Moschus), but for Bingpian (Borneolum Synthcticum) it was opposite. It is indicated that they have different effects on  $P_2$  and  $Q_2$  of *E. coli* in the second exponential growth phase, and have differences in warm and cold natures.

**Conclusion:** The microcalorimetry can accurately and objectively appraise differences of the drug property of Shexiang (Moschus) and Bingpian (Borneolum Synthcticum) and verify the objectivity and authenticity of the drug property, so as to provide a new and useful method for studies of the drug property of Chinese drugs.

**Keywords:** Shexiang and Bingpian; biothermodynamics; properties of Chinese drugs

The four natures of Chinese drugs, also described as the four “Qi” natures, first recorded in *Shennong Bencao Jing* (神农本草经 Shen Nong’s Herbal Classic), “the drugs have cold, hot, warm, cool in nature”.<sup>1</sup> The drug property is the core of Traditional Chinese medicine theories. It is not only a vinculum connecting Chinese Medicine with Chinese drugs, but also the basis of syndrome differentiation and treatment; It is also a outstanding mark of distinguishing Chinese crude drugs from plant drugs, natural drugs. In the four nature theory, the cold, hot natures are regarded as the primary drug property of Chinese drugs, “treating the cold-syndrome with hot-natured drugs, heat-syndrome being treated with drugs of cold or cool nature” is the most important therapeutic principle of traditional Chinese medicine. However, do the cold and hot natures objectively exist? What is its essence? How to measure the drug nature with the modern technology? Up to now, a substantial progress has not made in these researches yet.

In recent years, professor XIAO Xiao-he, et al. combine thermodynamics theory with the drug property, and proposed “traditional Chinese medicine thermodynamics theory”, and establish a method for assessment of cold-hot drug property based on analysis of biother-

modynamics, which provides a new method and research model for investigating Chinese drug property. The definition of the drug property based on the modern knowledge includes two means: one is that the medicine itself contains substances of different forms or different magnitudes of energy which can bring about physiological energy transference and heat changes in the body. The other is that the medicine possibly contains endogenous pyretogenic substances and relative substances, which generate a series of physiological or pathological reaction when acting on the human body.<sup>2-5</sup> The interaction in vivo is possibly physical or chemical reaction with transference or change of the energy, manifesting as absorption of heat or emission of heat.<sup>6,7</sup> The mechanism of the drug property action may be due

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to the delicate variation in energy. The microcalorimetry can accurately describe the small change of quantity of heat in the process with exact, objective, real-time, dynamic, on line and high-flux characteristics,<sup>8-13</sup> and it has already been used to describe the drug property, evaluate the drug quality, sieve the materials with pharmacodynamic action and study on the regularity of compatibility of drugs in a compound prescription and so on.<sup>14-21</sup>

Shexiang (Moschus) is an indispensable principal ingredient in many Chinese patent medicines such as Angong Niu Huang Pill (安宫牛黄丸) and Jufang Zhibao Pill (局方至宝丹), and it has extensive clinical application. Traditional Chinese medicine theory considers that Shexiang (Moschus) is a drug pungent in flavor and warm in property, and has the effects of inducing resuscitation and refreshment, promoting blood flow and clearing channels, detumescence and alleviating pain.<sup>1</sup> Both Bingpian (Borneolum Synthcticum) and Shexiang (Moschus) are drugs inducing resuscitation by means of aromatics. However, Bingpian (Borneolum Synthcticum) is a drug mild cool in nature. It is suitable for treatment of febrile diseases.<sup>1</sup> That is to say, they have different drug properties and different effects. In this paper, Shexiang (Moschus) and Bingpian (Borneolum Synthcticum) were studied by the microcalorimetry with *Escherichia coli* (*E. coli*) used as test bacteria, and the characteristic parameters such as growth rate constant ( $k$ ), maximum output power ( $P_m$ ) peak time ( $t_p$ ) and total heat-output ( $Q_t$ ) were used to evaluate their drug property differences, so as to verify the objectivity and authenticity of the drug property of Chinese medicines, and provide experimental basis and reference for establishing thermodynamics evaluation method of four properties of Chinese medicines, and also provide experimental and theoretical basis for clinical therapeutic principle of "treating the cold-syndrome with hot-natured drugs, heat-syndrome being treated with drugs of cold or cool nature".

## METHODS

### Instruments

The TAM Air 8-channels heat conduction isothermal microcalorimeter (Thermometric AB, Sweden);<sup>22</sup> YXQ LS-B full-automatic standing electrothermic steam sterilizer (Shanghai Jiangtou Instruments Co., Ltd, China); BS210s electronic balance (Beijing Sartorius Co., Ltd, China), KQ-500B ultrasonic washer (Kunshan City Ultrasonic Instruments Co., Ltd, China).

### Materials

*E. coli* (AB91113) was provided by the Chinese Center for Microorganism Culture, China.

Lactose broth (LB) culture medium: 5 g NaCl, 10 g peptone and 5 g yeast extract were dissolved in 1000 mL

deionised water (pH 7.2-7.4), and sterilized in high pressure steam at 121 °C for 30 min, and kept in a refrigerator at 4 °C after cooling for conservation.

Shexiang (Moschus) was purchased from the Dujiangyan Research Institute for Raising Musk-deer in Sichuan Province. Bingpian (Borneolum Synthcticum) was purchased from the Hehuachi Chinese Crude Drug Market in Chengdu. Both of them were identified by professor XIAO Xiao-he from 302 Military Hospital of China.

Preparation of the medicinal solution: In the sterile environment, powder of Shexiang (Moschus) and Bingpian (Borneolum Synthcticum) were dissolved in absolute ethyl alcohol and prepared different concentrations and kept in a freezer at 4 °C for conservation.

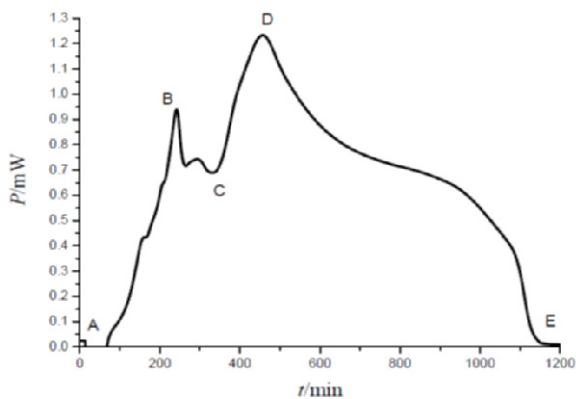
### Methods

The ampoule method was used,<sup>23</sup> under homothermal (37 °C), anoxic and sterile condition, 10 mL sterile deionized water added into a ampoule of 20 mL was used reference. After the baseline stabilized, *E. Coli* solution was inoculated in the LB culture medium and a suspension with the density of  $1 \times 10^6$  cells/mL was prepared. Then 10 mL of the suspension was taken and added into every ampoule with different concentrations of Shexiang (Moschus) and Bingpian (Borneolum Synthcticum) solutions respectively, meanwhile, setup the blank control group (*E. coli* solution without samples), and all the ampoules were sealed up and put into test channels of the microcalorimeter. After about 30 min (the temperature of ampoules reached at 37 °C), the power-time curves of *E. Coli* growth metabolism were recorded with PicoLog TC-80 work station until the recorder returned to the baseline, the experiment ended. The data were processed with OriginPro7.5 software and the bio-thermodynamics parameters such as growth rate constant ( $k$ ), maximum output power ( $P_m$ ) peak time( $t_p$ ) and total heat-output ( $Q_t$ ) were calculated. Then the software of Windows SAS8.0 was used for searching the chief parameters which are closely related with the effects of Shexiang (Moschus) and Bingpian (Borneolum Synthcticum) on *E.coli* growth metabolism.

## RESULTS

### Growth Rate Constant $k$

At 37 °C, the growth metabolism thermogenic curve of *E.coli* with no drug (blank sample) added was shown in Figure 1. In the anoxic and nutrition supply limited state and in combination with the general rule of the microorganism growth metabolism, this typical power-time curve of *E. coli* could be divided into four phases: growth phase (A-B), lag phase (B-C), stationary phase (C-D), and decline phase (D-E).<sup>24</sup>



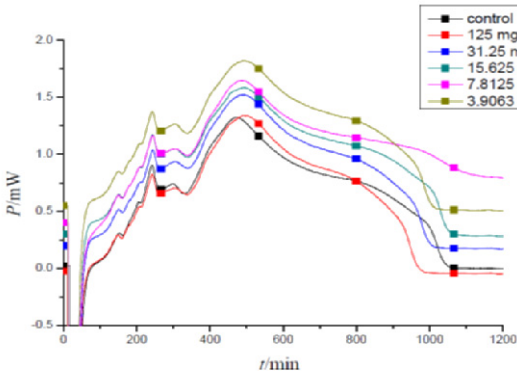
**Figure 1.** The power-time curve of *E. coli* growth metabolism.

In the growth phase, most researcher use exponential growth model and the equation  $P_t=P_0\cdot exp\left( kt \right)$  or  $\ln \left( P/P_0 \right) = kt^{25}$ , in which  $t$  is the time,  $P_t$  represents the calorimetric power at time  $t$ ,  $P_0$  represents that at time  $t_0$ , and  $k$  is the growth rate constant. Using this equation, the growth rate constant  $k$  was calculated.  $k$  is one of the characteristic parameters in microorganism growth. When different drugs or different concentrations of the drug are added under the same condition,  $k$  can be used for evaluating the activity of the drug.

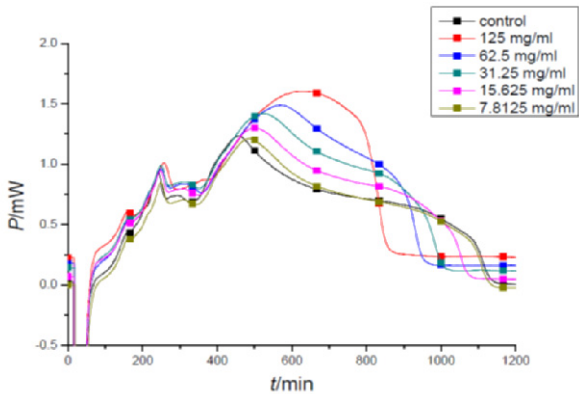
**The Biothermodynamics Parameters of *E. coli* affected by Different Concentrations of Shexiang (Moschus) and Bingpian (Borneolum Synthcticum)**

According to the above-mentioned method, the growth metabolism curves of *E. coli* affected by 5 different concentrations of Shexiang (Moschus) and Bingpian (Borneolum Synthcticum) and a blank control were determined in proper order, as shown in Figure 2 and Figure 3. And the bio-thermodynamics parameters such as  $t_1$ ,  $k_1$ ,  $P_1$ ,  $Q_1$  (the parameters at the first exponential

growth phase),  $t_2$ ,  $k_2$ ,  $P_2$ ,  $Q_2$  (the parameters at the second exponential growth phase ) were showed in Table 1.



**Figure 2.** The power-time curves of *E. coli* growth in the different concentrations of Shexiang (Moschus) (37°C, LB medium).



**Figure 3.** The power-time curves of *E. coli* growth in the different concentrations of Bingpian (Borneolum Synthcticum) (37°C, LB medium).

**Table 1.** The biothermodynamics parameters of *E. coli* growth in Shexiang (Moschus) and Bingpian (Borneolum Synthcticum) solutions

| Sample   | Content<br>(mg/mL) | $t_1$ /min | $t_2$ /min | $k_1$<br>( $\times 10^{-4} \text{ min}^{-1}$ ) | $k_2$<br>( $\times 10^{-5} \text{ min}^{-1}$ ) | $P_1$ /mW | $P_2$ /mW | $Q_1$ /J | $Q_2$ /J | $Q_t$ /J | $I/\%$ |
|----------|--------------------|------------|------------|------------------------------------------------|------------------------------------------------|-----------|-----------|----------|----------|----------|--------|
| Shexiang | 0.00               | 241.3      | 473.6      | 2.17                                           | 9.43                                           | 0.904     | 1.321     | 6.99     | 35.67    | 42.66    | -      |
|          | 3.9063             | 242.7      | 491.5      | 1.82                                           | 9.07                                           | 0.875     | 1.219     | 16.58    | 26.53    | 43.11    | 3.82   |
|          | 7.8125             | 244.5      | 484.9      | 2.44                                           | 8.25                                           | 0.763     | 1.246     | 11.47    | 32.90    | 44.37    | 12.51  |
|          | 15.625             | 243.6      | 493.2      | 1.80                                           | 7.21                                           | 0.922     | 1.306     | 12.12    | 33.58    | 45.70    | 23.54  |
|          | 31.25              | 244.9      | 490.5      | 1.78                                           | 6.81                                           | 0.887     | 1.371     | 9.09     | 35.09    | 44.18    | 27.78  |
|          | 125.00             | 242.1      | 489.4      | 1.87                                           | 6.69                                           | 0.875     | 1.388     | 7.35     | 35.77    | 43.22    | 29.06  |
| Bingpian | 0.00               | 241.7      | 455.8      | 1.77                                           | 10.00                                          | 0.940     | 1.233     | 8.42     | 36.20    | 44.62    | -      |
|          | 7.8125             | 249.6      | 485.6      | 1.83                                           | 8.02                                           | 0.850     | 1.214     | 7.68     | 35.56    | 43.24    | 19.80  |
|          | 15.625             | 248.2      | 500.4      | 1.54                                           | 6.62                                           | 0.899     | 1.253     | 10.24    | 34.95    | 45.19    | 33.80  |
|          | 31.25              | 249.4      | 523.6      | 1.71                                           | 6.24                                           | 0.892     | 1.320     | 10.82    | 34.26    | 45.08    | 37.60  |
|          | 62.50              | 248.5      | 566.7      | 1.95                                           | 5.65                                           | 0.813     | 1.341     | 10.65    | 32.26    | 42.91    | 43.50  |
|          | 125.00             | 256.1      | 627.3      | 2.11                                           | 4.34                                           | 0.814     | 1.376     | 13.08    | 28.24    | 41.32    | 56.60  |

### Principal Component Analysis of Biothermodynamics Parameters of *E. Coli* affected by Shexiang (Moschus) and Bingpian (Borneolum Synthcticum)

In this research, the datas were dealt with by principal component analysis (PCA). The eight quantitative parameters  $t_1$ ,  $t_2$ ,  $k_1$ ,  $k_2$ ,  $P_1$ ,  $P_2$ ,  $Q_1$ ,  $Q_2$  were expressed with X1-X8 in order. On the basis of eigenvalue >1, the accumulative contribution ratio of the four principal components accounting for 97.84% of the total variance were considered significantly. According to the eigenvectors, the relationship equations of the first four principal components expressed by standardized variables were showed in the following:

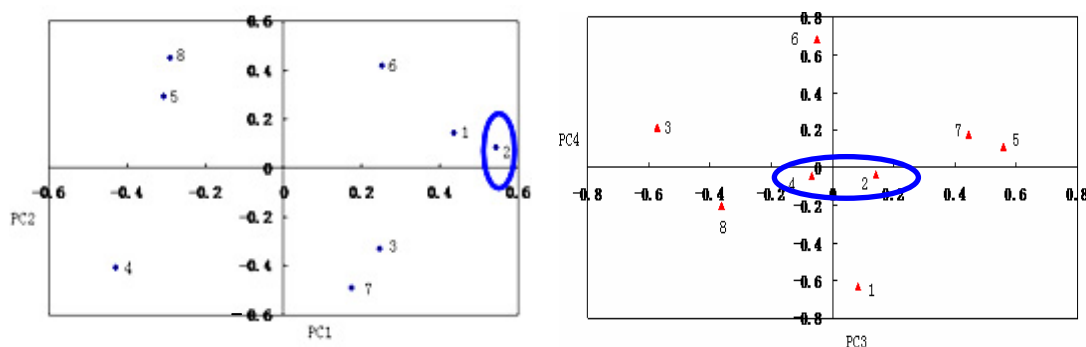
$$Z1=0.4361X1+0.5429X2+0.2446X3-0.4286X4-0.3061X5+0.2514X6+0.1732X7-0.2933X8;$$

$$Z2=0.1415X1+0.0835X2-0.3297X3-0.4024X4+0.2898X5+0.4202X6-0.4881X7+0.4513X8;$$

$$Z3=0.0830X2+0.1410X1-0.5721X3-0.0673X4-0.5578X5-0.0497X6+0.4437X7-0.3621X8;$$

$$Z4=-0.6339X1-0.0408X2+0.2150X3-0.0486X4+0.1125X5+0.6821X6+0.1739X7-0.1992X8.$$

The score plots (Figure 4) of the first four principal components showed that the parameters 2 ( $t_2$ ) and 4 ( $k_2$ ) possibly were the main evaluation indexes for appraising the action of Shexiang (Moschus) and Bingpian (Borneolum Synthcticum) on *E. coli* growth. The main parameters 2 ( $t_2$ ) and 4 ( $k_2$ ) were marked with blue circles. From the values of 2 ( $t_2$ ) and 4 ( $k_2$ ), it could be quickly and clearly found that the thermodynamic parameter  $t_2$  prolonged, and  $k_2$  decreased with the increase of Shexiang (Moschus) and Bingpian (Borneolum Synthcticum) concentrations, indicating that they inhibit the growth metabolism of *E. coli* with obvious concentration dependent relation.

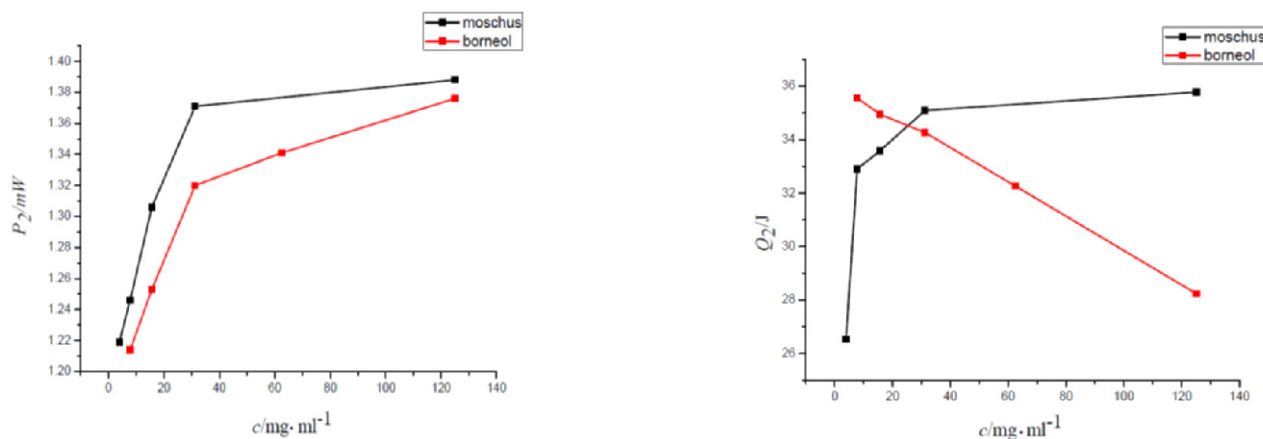


**Figure 4.** Scores plot for PCA. The scatter plot was obtained by PCA on the eight quantitative thermokinetic parameters (1)  $t_1$ , (2)  $t_2$ , (3)  $k_1$ , (4)  $k_2$ , (5)  $P_1$ , (6)  $P_2$ , (7)  $Q_1$ , (8)  $Q_2$  taken from the metabolic power-time curves of *E. coli* growth using software of Origin Pro7.5.

### Relation of Shexiang (Moschus) and Bingpian (Borneolum Synthcticum) Concentrations with $P_2$ and $Q_2$

As showed in Table 1 and Figure 5,  $P_2$  increased with the increase of Shexiang (Moschus) and Bingpian (Borneolum Synthcticum) concentrations,  $P_2$  affected by Shexiang (Moschus) being higher than that of Bingpian (Borneolum Synthcticum) within a concentration of

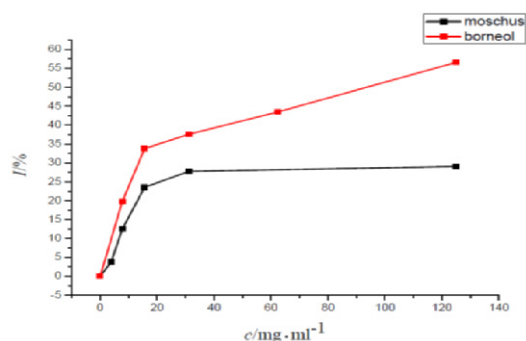
3.91–125.00 mg/mL;  $Q_2$  increased with the raising of Shexiang (Moschus) concentration; however  $Q_2$  decreased with increase of the concentration of Bingpian (Borneolum Synthcticum). Therefore, the chief characteristic thermodynamic parameters  $P_2$  and  $Q_2$  can be used to differentiate cold and hot properties of Shexiang (Moschus) and Bingpian (Borneolum Synthcticum).



**Figure 5.** Relationships of  $P_2$  and  $Q_2$  with different concentrations of Shexiang (Moschus) and Bingpian (Borneolum Synthcticum).

### Bacterial Growth Inhibitory Ratio

The bacterial growth inhibitory ratio ( $I\%$ ) was defined as:  $I\% = ((k_0 - k_c) / k_0) \times 100\%$ <sup>11</sup>, here,  $k_0$  was the rate constant of the blank control group,  $k_c$  was the rate constant for bacterial growth affected by the drug at a concentration of  $c$ . The inhibitory ratio is an important parameter for evaluating the anti-bacterial activity of a drug. From the data shown in Table 1, it can be seen that the inhibitory effect of Shexiang (Moschus) on the bacterial growth was weaker than that of Bingpian (Borneolum Synthcticum).



**Figure 6.** The inhibiting ratio of Shexiang (Moschus) and Bingpian (Borneolum Synthcticum) on bacterial growth.

### DISCUSSION

The main active component of Shexiang (Moschus) and Bingpian (Borneolum Synthcticum), muskone and borneol, is difficult to dissolve in water, but it can dissolve in ethyl alcohol, so, in the study both of them were dissolved in the absolute ethyl ethanol. In a preliminary experiment, the effect of different volumes of absolute ethyl alcohol on *E. coli* growth metabolism was firstly investigated so as to eliminate the influence of this solvent on the study result. The results showed that 30  $\mu$ L of ethanol had no obvious effect on growth and metabolism of *E. coli*. So, 30  $\mu$ L of Shexiang (Moschus) or Bingpian (Borneolum Synthcticum) solution was selected in the study.

In the past few years, the researchers have already been undertaking some beneficial explorations about the main material base and biological effect of the drug property, and the relationship of drug effect with the drug property and so on.<sup>26-30</sup> However, the results still can not well explain the essence of the drug property. The organism itself is a complex thermodynamic system, and possibly, the essence of the drug property is manifestations of interaction of the Chinese medicine with the organism itself.<sup>6</sup> Therefore, energy changes in the interaction course are monitored and recorded by using microcalorimetry, so as to get the relative thermodynamic parameters and the growth metabolism power-time curves of bacterial (*E. coli*) affected by drugs, and objectively, accurately, qualitatively and quantitatively evaluate cold or hot nature of a drug.

In the present study, cold and hot natures of Shexiang (Moschus) and Bingpian (Borneolum Synthcticum) were investigated by microcalorimetry. The windows SAS8.0 software was used to analyze the relative characteristic parameters, and in combination with detection of the thermogenic curves of growth metabolism of the tested microorganism affected by Shexiang (Moschus) and Bingpian (Borneolum Synthcticum), it was found that the two drugs had different effects on the metabolic heat-output of *E. coli*, which increased with increase of Shexiang (Moschus) concentration, and it was opposite for Bingpian (Borneolum Synthcticum). It is indicated that Shexiang (Moschus) and Bingpian (Borneolum Synthcticum) are different in the cold-hot natures. This is identical with traditional cognition of Chinese medicine about the properties of Shexiang (Moschus) and Bingpian (Borneolum Synthcticum), i.e., Shexiang (Moschus) is warm and Bingpian (Borneolum Synthcticum) is cold in nature. At the same time, it is also consistent with the results of investigation about the pharmacological action of four natures of Chinese medicines, the warm-and hot-natured Chinese drugs can raise excitability of the central nerve system, promote respiratory, circulatory and metabolic activities, increase the quantity of heat in body, enhance the resistance of the organism against pathogenic stimulus. Whereas the cold and cool natured drugs are opposite.<sup>31-33</sup> Therefore, the cold and hot properties of Shexiang (Moschus) and Bingpian (Borneolum Synthcticum) can be differentiated mainly by the two characteristic parameters  $P_2$  and  $Q_2$  of *E. coli* at the second exponential growth phase.

To sum up, in the study, the drug properties of Shexiang (Moschus) and Bingpian (Borneolum Synthcticum) were objectively verified by micro-calorimetry from biothermodynamics point of view, which provides a basis of the clinical medication principle of "treating the cold-syndrome with hot-natured drugs, heat-syndrome being treated with drugs of cold or cool nature". Of course, there was some shortages in this research. In future, it is necessary to thoroughly research the theory system of drug nature, including five tastes, raising, lowering, floating and sinking, toxicity, meridian distribution, etc., and establish a set of evaluation model and method system coinciding with traditional Chinese medicine theory, so as to better guide clinical medication.

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